

# Pith Necrosis of Tomato in Russia

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## Abstract

Tomato (*Solanum esculentum*), a major vegetable crop in Russia, is affected by several bacterial pathogens causing similar disease, known as pith rot. The disease is harmful in glasshouses and can be caused by *Pseudomonas corrugata*, *P. mediterranea*, *P. viridiflava*, and *P. fluorescens*. A survey of tomato plants in Russian Federation conducted in 2006 revealed the widespread presence of pith rot disease in North-west, Central, and South-eastern regions of Russia. One hundred two samples of diseased plants were collected in glasshouses. Isolations were made from plants with early symptoms of wilting from vessels above the ground level. Thirty five strains isolated from samples (58% of tested sites) presumptively identified as *P. corrugata*. The pathogenicity and physiologic properties of the strains were highly variable. Besides *P. corrugata*, 110 strains of other pseudomonades were isolated from the tested samples. Biochemical properties of the strains were highly diverse. However, almost 40% of the strains were recognized as *P. viridiflava* when others were assumed to be different biotypes of *P. fluorescens*. The bacteria were highly aggressive on tomato seedlings following inoculation by stem clipping.

## INTRODUCTION

Tomato (*Solanum esculentum*), a major vegetable crop in Russia, is affected by several bacterial pathogens causing similar disease, known as pith rot. The disease is harmful in glasshouses and can be caused by *Pseudomonas corrugata* Roberts et Scarlett (Westcott and Horst, 2001), *P. mediterranea*, *P. viridiflava* and *P. fluorescens* (Saygili et al., 2004).

Pith rot was found in Russia for the first time in 1989 on plants grown from imported seeds in glasshouses at Moscow, Saratov and Kemerovo regions (Popkova and Nosova, 1989). It was most harmful in mixed infection with *C. michiganensis* subsp. *michiganensis*. At 2006-2007 symptoms of pith rot were observed with different severity in most of glasshouses in Moscow, Leningrad, Nizhniy Novgorod, Tver, Pskov, Saratov, Voronezh, Volgograd region, and Tatarstan. We evaluated frequency of potential pith rot pathogens in those regions.

## MATERIALS AND METHODS

### Isolation of Bacteria

Fluorescent and non-fluorescent pseudomonades were isolated from diseased plant collected in the assayed regions on semi-selective and non-selective media (Laboratory guide for identification of plant pathogenic bacteria, 2001). Bacteria were evaluated for Gram-staining, oxidase, ADH, growth on KB, YDC, and other physiologic tests, including utilization of 16 carbohydrates.

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## PCR Analysis

To confirm the identification of *P. corrugata* PCR analysis was made with primers (PC5/1 and PC5/2) for 1100 bp fragment (Catara et al., 2002), and with BOX A primer for characteristic patterns in comparison to reference strain ICMP 5819. Identity of *P. viridiflava* was identified by PCR-RFLP assay as described elsewhere (Jakob et al., 2002).

## Pathogenicity Identification

Bacterial cultures were grown in liquid NBY overnight, and adjusted to  $1 \times 10^6$  cfu/ml. For HR leaves of geranium, plectranthus, and tobacco plant leaves were injected and reaction was observed during 24h following the inoculation. For host pathogenicity seedlings of susceptible tomato cv. 'Dubok' were injected in stem. After inoculation, plants were covered by polyethylene bags for 3 days to maintain high humidity. Symptoms were observed after 14 days on plants inoculated with virulent bacteria.

## RESULTS AND DISCUSSION

Sixty samples of diseased plants were collected in glasshouses. Isolation of bacteria was made from plants with early symptoms of wilting from vessels above the ground level. Thirty five strains isolated from samples in 58% of tested sites were presumptively identified as *P. corrugata* based on their positive HR, colony morphology and biochemical properties, including positive oxidase, blue pigmentation, and poly-b-oxybutyrate accumulation. The pathogenicity test has confirmed the identity of the strains. Brown water-soaked and dry pith necrosis of the stem was observed in 14 days on tomato seedling inoculated with *P. corrugata*.

These strains were compared to 5 isolates obtained in Russia since 1989 and type strain of *P. corrugata*. The pathogenicity and physiologic properties of the strains were variable. Considerable difference was found in carbohydrates utilization, BOX A-PCR profiles and virulence on tomato plants (data not shown).

Besides *P. corrugata*, 110 strains of other pseudomonades were isolated from the tested samples (Table 1). Biochemical properties of the strains were highly diverse. However, almost 40% of the strains were recognized as *P. viridiflava* when others were assumed to be different biotypes of *P. fluorescens*.

The bacteria identified as *P. fluorescens* caused HR on at least one indicator plant and were aggressive on tomato seedlings inoculated by stem clipping. Symptoms caused by the strains varied from local water-soaked lesion and soft rot to dry pith necrosis of the stem.

Pectolytic enterobacteria identified as *Erwinia carotovora* were present in 84% of tested samples in necrotic plant tissues.

## CONCLUSIONS

Pith stem necrosis - one of major bacterial disease of tomato Russia is associated with three pathogenic bacteria: *Pseudomonas fluorescens*, *P. corrugata*, and *P. viridiflava*, present as mixed infection. The disease is spread at the same region as *Clavibacter michiganensis* subsp. *michiganensis*, and probably more harmful on plants with early stage of tomato canker development. Seed of tomato must be tested for *Pseudomonas corrugata* as possible seed-born pathogen.

## ACKNOWLEDGEMENTS

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## Tables

Table 1. Identification of pathogens in 102 diseased tomato samples with pith necrosis symptoms by isolation and PCR.

Pathogens	Samples positive for target bacteria by phenotypic tests	# Strains isolated (strains PCR-positive)
<i>P. corrugata</i>	35 (35%)	42 (35)
<i>P. viridiflava</i>	18 (18%)	44 (40)
<i>P. fluorescens</i>	59 (58%)	66 (-)
<i>Erwinia carotovora</i>	86 (84%)	-